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Syndecan-1: A Quantitative Marker for the Endotheliopathy of Trauma

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BACKGROUND: Endothelial glycocalyx breakdown elicits syndecan-1 shedding and endotheliopathy of trauma (EoT). We hypothesized that a cutoff syndecan-1 level can identify patients with endothelial dysfunction who would have poorer outcomes.

STUDY DESIGN: We conducted a prospective observational study. Trauma patients with the highest level of activation admitted from July 2011 through September 2013 were eligible. We recorded demographics, injury type/severity (Injury Severity Score), physiology and outcomes data, and quantified syndecan-1 and soluble thrombomodulin from plasma with ELISAs. With receiver operating characteristic curve analysis, we defined EoT+ as the syndecan-1 cutoff level that maximized the sum of sensitivity and specificity (Youden index) in predicting 24-hour in-hospital mortality. We stratified by this cutoff and compared both groups. Factors associated with 30-day in-hospital mortality were assessed with multivariable logistic regression (adjusted odds ratios and 95% CIs reported).

RESULTS: From receiver operating characteristic curve analysis (area under the curve = 0.71; 95% CI 0.58 to 0.84), we defined EoT+ as syndecan-1 level ≥40 ng/mL (sensitivity = 0.62, specificity = 0.73). Of the 410 patients evaluated, 34% (n = 138) were EoT+ patients, who presented with higher Injury Severity Scores ($p < 0.001$) and blunt trauma frequency ($p = 0.016$) than EoT− patients. Although EoT+ patients had lower systolic blood pressure (median 119 vs 128 mmHg; $p < 0.001$), base excess and hemoglobin were similar between groups. The proportion of transfused (EoT+ 71.7% vs EoT− 36.4%; $p < 0.001$) and deceased EoT+ patients (EoT+ 24.6% vs EoT− 12.1%; $p < 0.001$) was higher. EoT+ was significantly associated with 30-day in-hospital mortality (adjusted odds ratio = 2.23; 95% CI 1.22 to 4.04).

CONCLUSIONS: A syndecan-1 level ≥40 ng/mL identified patients with significantly worse outcomes, despite admission physiology similar to those without the condition. (J Am Coll Surg 2017;225: 419−427. © 2017 The Authors. Published by Elsevier Inc. on behalf of the American College of Surgeons. This is an open access article under the CC BY-NC-ND license [http://creativecommons.org/licenses/by-nc-nd/4.0/].)

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Hemorrhage is the leading cause of early and potentially preventable mortality in trauma patients, with 58% of these deaths occurring in the first 3 hours after hospital admission.[1] Hemorrhagic shock is the underlying mechanism responsible for this high mortality rate because it leads to exsanguination, inflammation, coagulopathy, endothelial dysfunction, and an increase in vascular permeability.[2,3] The endothelial glycocalyx (EGL) is a negatively charged complex layer of proteoglycans and glycoproteins on top of endothelial cells of which syndecans and hyaluronic acid are major components. This structure is responsible, in part, for the maintenance of vascular permeability.[4] Loss of the integrity of the EGL increases vascular permeability, leading to capillary leak,

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Abbreviations and Acronyms

ED = emergency department
EGL = endothelial glycocalyx
EoT = endotheliopathy of trauma
IQR = interquartile range
ISS = Injury Severity Score
ROC = receiver operating characteristic
sTM = soluble thrombomodulin

and results in exposure of endothelial cells to circulating platelets and leukocytes, which initiates an inflammatory response and alters coagulation.[3,5] Damage and thinning of the EGL have been linked to the deleterious effects of reperfusion damage (ie after post-cardiac arrest syndrome) and to other inflammatory states, such as hyperglycemia in diabetes mellitus.[6-8] Endothelial glycocalyx breakdown also disturbs blood flow-induced forces, such as shear stress and wall tension, due to the essential role that EGL plays as a mechanosensor.[9] Therefore, EGL breakdown can be the pivotal point where the downstream effects of trauma interact and lead to endothelial dysfunction, coagulopathy, edema, and organ dysfunction, which ultimately results in poor outcomes.[2,10] The systemic effects of these responses comprise the syndrome called the "endotheliopathy of trauma" (EoT).[11] Clinically, there is not yet a readily available method to quantify endothelial damage, but previous studies have shown a strong association between the shedding of EGL components—mainly syndecan-1—and coagulopathy, edema, and mortality.[2,5,10] Syndecan-1 (a heparan-sulfate proteoglycan expressed in both endothelial and epithelial cells) has been widely studied in relation to traumatic injury and is currently considered a main marker of EGL breakdown.[2,10,12,13] Thrombomodulin is an anticoagulant protein expressed on the endothelial surface and plays a role in the activation of the protein C anticoagulant pathway.[4,14-16] After trauma, the increase of the inflammatory cytokines tumor necrosis factor α and interleukin 6 results in downregulation of thrombomodulin and activation of neutrophils that can cleave thrombomodulin, releasing it into the circulation as soluble thrombomodulin (sTM), a less active form and a well-known marker of endothelial cell injury.[14-17]

Hemorrhagic shock induces shedding of syndecan-1, facilitating the exposure of the injured endothelium to pro-inflammatory mediators and altering its integrity, which results in increased permeability.[10,18] Recently, Johansson and colleagues demonstrated an association between sympathoadrenal activation, inflammation, coagulopathy, and shedding of syndecan-1.[2,3,19,20] Other studies have also shown the association between high circulating levels of syndecan-1 and increased endothelial permeability, which correlated with higher transfusion volumes and worse outcomes.[10] In addition, the protective effects of therapies that target EoT and EGL, such as plasma-based resuscitation, seem to be mediated in part by the modulation of syndecan-1, as demonstrated by in vitro studies.[5,21,22] Similarly, in a rodent model, Haywood-Watson and colleagues[5] showed that fresh frozen plasma repairs the EGL, restores endothelial syndecan-1, and modulates inflammation.

Syndecan-1 seems to be involved in several of the downstream effects after traumatic injury that lead to the EoT.[3,10,19,23] The aim of this study was to determine a quantitative index of EoT using circulating levels of syndecan-1, a biomarker of EGL breakdown. We hypothesized that there is a cutoff level of shed syndecan-1 that allows the identification of trauma patients with endothelial dysfunction, at risk of progressing into EoT, and who would have an increased need for blood transfusions and poorer outcomes.

METHODS

Study design and analysis sample

This prospective observational study was conducted at the Texas Trauma Institute Memorial at Hermann Hospital Texas Medical Center, a Level I trauma center, and The University of Texas Health Science Center at Houston. Earlier approval was obtained from The University of Texas Health Science Center at Houston IRB (HSC-GEN-12-0059). The current article reports additional analysis of data from 410 patients (14 patients were excluded because of missing essential laboratory and clinical data) of the 424 patients previously reported by Johansson and colleagues.[20] As we limited our patient data to having no missing values, there are minor differences between the articles. We included adult trauma patients admitted between July 2011 and September 2013 who met criteria for the highest level of trauma team activation. Consent was obtained from the patient or a legally authorized representative within 72 hours of admission. We obtained a waiver of consent for those patients discharged or who died within 24 hours of admission. We excluded cases where patients were younger than 18 years, pregnant, prisoners, and enrolled in other studies; patients who declined to give consent (their blood samples destroyed); and those from whom we could not obtain an initial blood draw. On emergency department (ED) arrival, 20 mL of blood was obtained, then transferred into Vacutainer tubes containing 3.2% citrate, and inverted to ensure proper anticoagulation.

In this study, we collected blood samples from 560 patients. Only patients suffering from blunt or penetrating trauma with available data on Injury Severity Score (ISS) and syndecan-1 levels were included in the analysis. This resulted in the exclusion of 150 patients (Fig. 1). The excluded patients did not differ significantly from the population of this study: median age was 40 years (interquartile range [IQR] 27 to 50 years), 78% were male, and in-hospital mortality was 14.8%. Designated study personnel prospectively collected patient demographics, vital signs, standard laboratory values, transfusions data, weighted revised trauma scores, and mechanisms and severity of injuries at the time of admission. Outcomes and complications data were obtained from medical records.

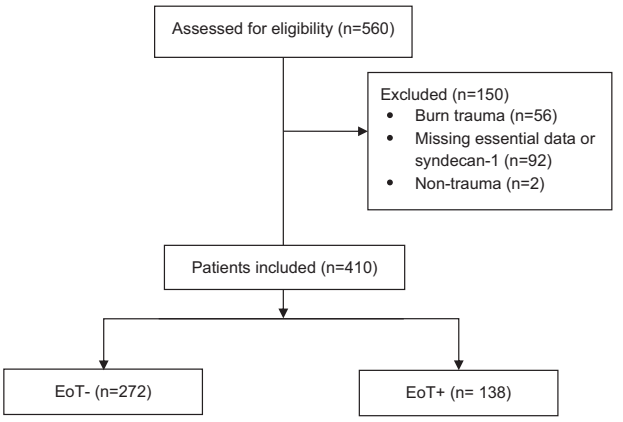

Figure 1. CONSORT (Consolidated Standards of Reporting Trials)-like diagram showing the selection process used to identify trauma patients for inclusion in the study. EoT, endotheliopathy of trauma.

ELISAs

Plasma obtained at baseline (on admission to the ED) was used to quantify levels of syndecan-1 using commercially available immunoassays (syndecan-1; Diaclone SAS; lower limit of detection 4.94 ng/mL), and sTM as a marker of endothelial cell injury (sTM; Nordic Biosite; lower limit of detection 0.31 ng/mL) according to manufacturer recommendations.

Statistical analysis

Summary statistics were used to describe continuous variables (medians and IQRs reported throughout) and categorical data were presented as frequency and percentage. Comparisons between the EoT− and EoT+ study groups for continuous variables were assessed using 2-sample *t*-tests for normally distributed data and Mann-Whitney U or Kruskal-Wallis tests in case of non-normally distributed data. Chi-square tests were used for comparisons between categorical variables. Statistical significance was set at the $p < 0.05$ level. The correlation between syndecan-1 and sTM was explored with Spearman's rank correlation coefficient. The primary EoT biomarker of interest was on-admission syndecan-1 levels. We used receiver operating characteristic (ROC) curve analysis with the Youden index to determine the cutoff value of syndecan-1 that maximized the sum of sensitivity and specificity in predicting 24-hour in-hospital mortality. We chose mortality during the first 24 hours (counting from ED admission) as the end point for this ROC analysis; as has been reported in several studies,[1,24,25] most deaths in trauma patients occur during this time window. Therefore, it is during these first 24 hours that interventions that target the EoT and have the potential to improve outcomes should be implemented. We validated the obtained ROC curve with 3-fold cross validation. The inferred cutoff value was then used to classify patients into an EoT+ group based on a syndecan-1 level $\geq$40 ng/mL or an EoT− group those with levels below this cutoff.

Our main outcome was 30-day in-hospital mortality. Purposeful selection of covariates[26,27] was used to construct a multivariable logistic regression model for factors associated with 30-day in-hospital mortality. We predefined clinically relevant variables in hemorrhagic shock and trauma that are usually collected within 30 minutes of hospital arrival, such as laboratory parameters, vital signs, mechanism and severity of injury, as well as our biomarker of interest EoT+ (as defined). After the model building approach mentioned,[26,27] only variables with a p value < 0.2 in the univariate analysis were included in the preliminary model: age, EoT+ (as defined), ISS, blunt mechanism, ED systolic blood pressure, platelet count, sex, and base excess. After purposeful selection of covariates, we obtained 4 variables of significance, age, ISS, EoT+, and ED systolic blood pressure, which were entered into a multivariable logistic regression model for factors associated with 30-day in-hospital mortality. We also controlled for base excess because this variable, despite lacking statistical significance, had a significant effect in the presence of other variables. Results are presented as adjusted odds ratios with 95% CIs and p values for all covariates in the final model. The presented adjusted odds ratios are interpreted as changes in the odds per unit in the predictor for the continuous variables (age, ISS, ED systolic blood pressure, and base excess) after adjusting for the other variables in the model. All statistical analysis was conducted with commercially available software, STATA, version 13.0 (Stata Corp).

RESULTS

Syndecan-1 and sTM levels were measured from samples collected on ED arrival from 410 patients. Overall, 76.8% of these patients were male, with a median age of 40 years (IQR 27 to 55 years) (Table 1). Blunt trauma was the predominant mechanism of injury (71.5%) and patients were moderately injured (median ISS 17; IQR 9 to 26). Most patients were normotensive on ED admission with median systolic blood pressure of 124 mmHg (IQR 107 to 142 mmHg), slightly acidotic with a median pH of 7.32 (IQR 7.25 to 7.37), and had ED hemoglobin (median 13.1 g/dL) and platelet count levels (median 221 $\times$ 10^9/L) within normal ranges. The overall 30-day in-hospital mortality rate was 16.3% (Table 1), with nearly two-thirds of the deaths occurring within the 24 hours after hospital admission. Death on the first day of hospital admission was due to head injury (n = 24 [61.5%]), hemorrhage (n = 11 [28.2%]), and other causes, including cardiac arrest and respiratory failure (n = 4 [10.3%]).

Defining the endotheliopathy of trauma

Our primary biomarker of interest was circulating levels of syndecan-1 measured in blood samples collected on arrival. Initially, we explored differences in transfusion and 30-day in-hospital mortality rates across quartiles. We found that compared with the rest, patients with syndecan-1 levels in the upper quartile ($\geq$60 ng/mL) had the highest rates of blood transfusions and 30-day in-hospital mortality, 73.8% and 26.2%, respectively (both $p < 0.01$). In addition, syndecan-1 levels of nonsurvivors were significantly higher than those of survivors (median syndecan-1 level in survivors 24.7 ng/mL vs median syndecan-1 level in nonsurvivors 40.4 ng/mL; $p = 0.01$). At the cutoff level $\geq$60 ng/mL, the sensitivity and specificity to identify 24-hour in-hospital mortality were only 47.0% and 76.5%, respectively. To better identify a cutoff level of syndecan-1 to define EoT+, we constructed an ROC analysis with the Youden index (J) and 24-hour in-hospital mortality as the outcomes variable (area under the curve = 0.71; 95% CI 0.58 to 0.84; Fig. 2). With the Youden index, we determined our cutoff level (syndecan-1 $\geq$40 ng/mL) as the level of syndecan-1 that maximized the sum of sensitivity and specificity in predicting 24-hour in-hospital mortality (J = 0.35, sensitivity at cutoff = 0.62, specificity at cutoff = 0.73). We validated the fitted ROC curve analysis with 3-fold cross validation. Syndecan-1 was predictive of 24-hour in-hospital mortality in both the fitted model and in the cross-validated model (mean cross-validated area under the curve = 0.67; IQR 0.59 to 0.74) corroborating our findings.

After stratification by the optimal cutoff, patients defined as EoT+ (n = 138; syndecan-1 $\geq$40 ng/mL) were compared with patients EoT− (n = 272; syndecan-1 <40 ng/mL). Median syndecan-1 concentration was more than 6 times higher in EoT+ than in EoT− patients (EoT+ median 108 ng/mL vs EoT− median 17 ng/mL; $p < 0.001$). Although there were no differences in age or sex between groups, type of trauma, injury severity, and anatomical region of injury did differ significantly. EoT+ patients were more likely to suffer from blunt and chest trauma and were significantly more injured (EoT+ ISS median 21 vs EoT− ISS median 16) than EoT− patients (all $p < 0.05$). A detailed summary of median age, sex, blunt trauma, traumatic brain injury (Abbreviated Injury Scale Head >2), and chest trauma (Abbreviated Injury Scale Chest >2) proportions in both groups can be found in Table 1. When comparing vital signs and admission physiology between groups, we found that median ED systolic blood pressure was lower in EoT+ patients, but not in the hypotensive range. Hemoglobin and platelet median counts fell into normal ranges; however, median platelet count was significantly lower in EoT+ patients (Table 1).

Glycocalyx shedding and endothelial disruption

Soluble thrombomodulin levels in patients EoT+ were nearly 1.5 times higher (median 6.7 ng/mL) than those in the EoT− group (4.7 ng/mL) ($p < 0.001$). There was a positive moderate correlation between syndecan-1 and sTM levels (Spearman's ρ correlation coefficient = 0.50; 95% CI 0.42 to 0.56).

Transfusions and outcomes

Endotheliopathy of trauma-positive patients had worse outcomes than EoT− patients did. Most patients (71.7%) in the EoT+ group needed blood transfusions and required 4 times more units of blood products in the initial 24 hours than EoT− patients (Table 2). When comparing hospital length of stay, patients without the syndrome (EoT−) had nearly 2 times more hospital-free days than those in the EoT+ group (median 23 days; IQR 10 to 28 days and median 13 days; IQR 0 to 25 days, respectively). Similarly, median ICU-free days and ventilator-free days were also significantly lower in the EoT+ group than those in the syndrome-free group, EoT− (all $p < 0.0001$).

When we evaluated 30-day in-hospital mortality, we found a higher rate of mortality in EoT+ patients than in EoT− patients (Table 2). We constructed a multivariable logistic regression model with 5 covariates (EoT+, base excess, ISS, ED systolic blood pressure, and age) to evaluate factors associated with 30-day in-hospital

Table 1. Patient Characteristics, Mechanism and Severity of Injury, Admission Physiology, Endothelial Biomarkers, and Subgroup Comparisons in 410 Patients Admitted to a Level I Trauma Center

Variable	All patients	EoT−*	EoT+†	p Value
Demographic				
n	410	272	138	
Age, y, median (IQR)	40 (27 to 55)	39 (27 to 55)	41 (27 to 55)	0.86
Sex, n (%)				0.61
Female	95 (23.2)	61 (22.4)	34 (24.6)	
Male	315 (76.8)	211 (77.6)	104 (75.4)	
Injury type and severity				
Blunt trauma, n (%)	293 (71.5)	184 (67.6)	109 (79.0)	0.016
Injury Severity Score, median (IQR)	17 (9 to 26)	16 (9 to 25)	21 (10 to 30)	<0.001
AIS head ≥3, n (%)	179 (43.7)	120 (44.1)	59 (42.8)	0.79
AIS chest >2, n (%)	134 (32.7)	71 (26.1)	63 (45.7)	<0.001
Admission physiology and biochemistry, median (IQR)				
Systolic blood pressure, mmHg	124 (107 to 142)	128 (110 to 145)	119 (91 to 140)	<0.001
Heart rate, beats/min	92 (78 to 109)	92 (78 to 110)	92 (79 to 109)	0.99
Base excess, mEq/L	−3 (−6 to 1)	−2 (−5 to 1)	−3 (−7 to 1)	0.13
Platelet count, 10^9/L	221 (182 to 270)	225 (190 to 272)	211 (162 to 262)	0.005
Hemoglobin, g/dL	13.1 (11.7 to 14.4)	13.2 (11.9 to 14.4)	13 (11.2 to 14.5)	0.37
Glasgow Coma Scale score	11 (3 to 15)	12 (3 to 15)	7 (3 to 15)	0.43
Revised trauma score	6.2 (4.09 to 7.84)	6.9 (4.09 to 7.84)	6 (4.09 to 7.84)	0.044
Biomarker, ng/mL, median (IQR)				
Syndecan-1	13 (26 to 60)	17 (10 to 26)	108 (60 to 227)	
Soluble thrombomodulin	5.2 (3.8 to 7.2)	4.7 (3.5 to 6.1)	6.7 (5.4 to 9.4)	<0.001

Table includes data of 410 patients from the sample population presented previously by Johansson and colleagues.[20]
*Syndecan-1 <40 ng/mL.
†Syndecan-1 ≥40 ng/mL.
AIS, Abbreviated Injury Scale; EoT, endotheliopathy of trauma; IQR, interquartile range.

mortality. Although base excess was not significant, we included this variable in the fitted model because it had a significant effect in the presence of other variables. While controlling for these variables, we found that EoT+ was significantly associated with an increased likelihood of 30-day in-hospital mortality (adjusted odds ratio = 2.23; 95% CI 1.22 to 4.04). Other covariates associated with an increase in likelihood of 30-day in-hospital mortality were well-known predictors of mortality, that is, age, ISS, and ED systolic blood pressure (Table 3).

DISCUSSION

Endotheliopathy of trauma is a term proposed by Holcomb and Pati[11] in an attempt to characterize a syndrome that arises after injury, probably prompted by EGL breakdown. Likely, this pivotal event triggers the downstream responses that lead to systemic effects, such as coagulopathy, edema, organ-barrier dysfunction, and endothelial dysfunction. In this study, we demonstrated that syndecan-1 has a role not only as biomarker of EGL breakdown, but also as quantitative index for EoT.[3,10,11,13] A syndecan-1 concentration ≥40 ng/mL identified a group of patients with endothelial dysfunction (EoT) in the absence of clinically significant differences in admission physiology. Interestingly, like the results of previous studies,[1,24] 24-hour in-hospital mortality in our population was predominantly due to head injury, indicating that the observations reported in this study are generalizable to the trauma population, and not confined to patients suffering from severe hemorrhage.

To our knowledge, we are the first to report the use of syndecan-1 as a quantitative index of EoT, and the results presented in this article support its potential use as an early biomarker for EoT, especially given the lack of clinically significant differences in admission physiology between EoT+ and EoT− patients. Clinical studies have demonstrated elevated levels of syndecan-1 and sTM (markers of EGL breakdown and endothelial damage, respectively) in relation to trauma-induced coagulopathy.[2,3,14-17] Soluble thrombomodulin is also independently associated with increased 7-day and 28-day mortality in trauma patients,[20] and a higher

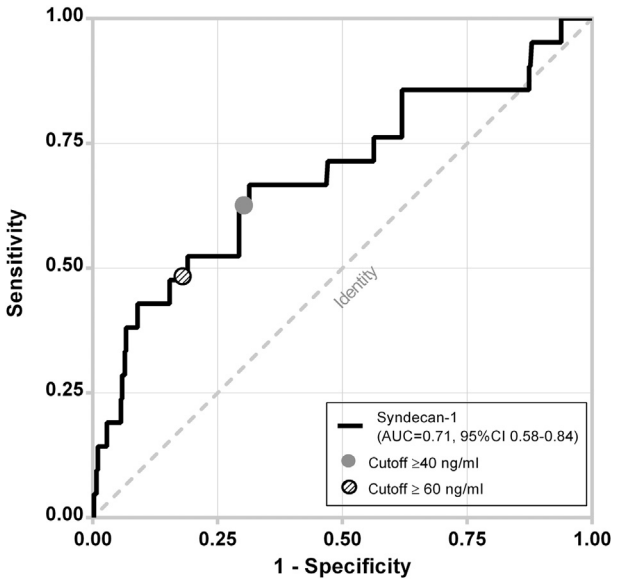

Figure 2. Receiver operating characteristic curve analysis and identification of the optimal cutoff with the Youden index (J) for syndecan-1 as predictor of 24-hour in-hospital mortality in 410 trauma patients. Patients were defined as having the endotheliopathy of trauma (EoT+) based on a syndecan-1 level ≥40 ng/mL selected with the Youden index (J = 0.35) as the cutoff value that maximized the sum of sensitivity and specificity in predicting 24-hour in-hospital mortality. The point that corresponds to the cutoff for the upper quartile of syndecan-1 levels (≥60 ng/mL) is also represented here. AUC, area under the curve.

risk of multiple organ failure and mortality in sepsis.[28] We explored the correlation between syndecan-1 and sTM levels in this population and found that, although significant, the strength of the correlation between these 2 biomarkers was only moderate. We can speculate that this is because, on admission, endothelial cell damage as evidenced by levels of sTM has not yet occurred.

The observed increased need for transfusion and higher mortality in the EoT+ group despite similar admission physiology among groups is important because this can reflect initial physiologic deterioration derived from EGL breakdown as demonstrated by syndecan-1 shedding. At this very early stage, there has not been adequate time to develop detectable physiologic derangements, but over time, the downstream effects of the original insult result in deterioration that could trigger an increased need for transfusion. Endothelial glycocalyx breakdown and syndecan-1 shedding triggers neurohormonal overactivation, coagulopathy, endothelial dysfunction, and increased permeability.[2,11,19,24,29] These systemic effects are potential therapeutic targets, which has prompted the study of interventions ranging from β-blockers to modulate the catecholamine surge to plasma to repair the endothelium.[29-34] Therapies such as plasma-based resuscitation seem to re-establish syndecan-1 on the cell surface and reduce shedding, while also decreasing endothelial hyperpermeability and modulating inflammation.[5,13,29,32,35,36] In a mice model, Ban and colleagues[21] demonstrated that syndecan-1 mediates the protective effect that plasma has on intestinal epithelium after hemorrhagic shock. In this study, we intended to define clearly what EoT is clinically by establishing a cutoff. Johansson and colleagues[20] recently showed that syndecan-1, but not sTM or sE-selectin (biomarkers of endothelial cell damage and activation, respectively) predicted <24-hour mortality in trauma patients. We show here that not only is EoT+ independently associated with 30-day in-hospital mortality, but EoT+ also identifies a group of patients with poorer clinical outcomes despite similar admission physiology among EoT+ and EoT− patients. Even though a sensitivity of 62.0% would result in a number of false positives, in acute settings such as trauma and hemorrhagic shock, where most deaths occurred on the first day of hospital arrival (and >25% within 1 hour of hospital arrival),[24] the benefits of interventions that could improve outcomes outweigh the risks. A quantitative marker of EoT provides an objective measure of EGL breakdown and endothelial damage, and could be used to assess the progression of EoT. Having indices of EoT would increase the clinician's index of suspicion, resulting in closer surveillance and monitoring of patients. More importantly, such a quantitative index helps to pave the way for studies that focus on investigating strategies for early clinical screening of EoT (in the ED or even the prehospital setting) and timely appropriate interventions that attempt endothelial repair.[2,10,19,20] Syndecan-1 can be used as a confirmatory marker, but currently its use as an early screening tool for EoT is less practical because it requires ELISA assays that are time consuming, so by the time results are available they are no longer relevant. Therefore, techniques and studies that focus on the acute clinical identification of EoT are needed. Previous work at our laboratory demonstrated that trauma patients with low plasmatic oncotic pressure (plasma colloid-osmotic pressure) had higher plasma levels of syndecan-1 and other glycocalyx components compared with those with normal levels of plasma colloid-osmotic pressure.[10,37] Additional in vitro experiments also showed an association between shedding of syndecan-1, higher levels of catecholamines, and increased permeability in these subjects.[10,36] These findings support the hypothesis that the alteration of capillary Starling's forces reflects EGL disruption and endothelial dysfunction that allow the translocation of proteins from the intravascular compartment to the interstitial space.[37,38]

Table 2. Blood Products and Crystalloid Use, Outcomes, and Mortality Comparisons Between Endotheliopathy of Trauma-Positive and Endotheliopathy of Trauma-Negative Patients

Variable	Total	EoT−*	EoT+†	p Value
n	410	272	138	
Transfusion				
Transfused, n (%)	198 (48.3)	99 (36.4)	99 (71.7)	<0.001
RBC, U, median (IQR)	0 (0−2)	0 (0−1)	2 (0−6)	<0.001
Plasma, U, median (IQR)	0 (0−2)	0 (0−1)	2 (0−6)	<0.001
Platelets, U, median (IQR)	0 (0)	0 (0)	0 (0−2)	<0.001
24-h total blood,‡ U, median (IQR)	0 (0−6)	0 (0−2)	4 (0−16)	<0.001
24-h total crystalloid volume, mL, median (IQR)	1,600 (0−3,700)	0 (0−2,600)	3,100 (300−5,800)	<0.001
Outcomes				
Hospital-free days, median (IQR)	21 (4−27)	23 (10−28)	13 (0−25)	<0.001
ICU-free days, median (IQR)	28 (16−30)	28 (21−30)	23 (0−29)	<0.001
Ventilator-free days, median (IQR)	29 (20−30)	29 (26−30)	28 (0−30)	<0.001
24-h mortality, n (%)	39 (9.5)	16 (5.9)	23 (16.7)	0.001
30-d in-hospital mortality, n (%)	67 (16.3)	33 (12.1)	34 (24.6)	0.001

Table includes data of 410 patients from the sample population presented previously by Johansson and colleagues.[20]
*Syndecan-1 <40 ng/mL.
†Syndecan-1 ≥40 ng/mL.
‡Total units of blood products (RBC, plasma, and platelet units).
EoT, endotheliopathy of trauma; IQR, interquartile range.

In addition to an increased need for transfusion of blood products, the rates of early (within the first 24 hours of hospital arrival) and late (at 30 days of hospital arrival) mortality in the EoT+ group were significantly higher than in the EoT− group. This difference in mortality could be due to EoT+ patients being more injured, as indicated by higher ISS. However, endothelial dysfunction could have been a contributing factor because a higher proportion of patients in the EoT+ group presented with Abbreviated Injury Scale Chest scores >2, implicating more endothelial damage (the lungs represent 7% of endothelial surface area in the body[39]), and consequently an increased risk of EoT.[40] A study by Wright and colleagues[40] demonstrated that hemodynamically stable patients that presented with pulmonary contusion on initial chest x-ray had a higher prevalence of coagulopathy that probably ensued from endothelial injury; this finding was also associated with a higher mortality rate in these patients. As demonstrated by Johansson and colleagues[2] and Ostrowski and colleagues,[3] endogenous heparinization can ensue from EGL breakdown and contribute to trauma-induced coagulopathy. Although in this study we did not assess the relationship between high syndecan-1 levels and altered coagulation parameters, in a recent study our collaborators investigated the association between endotheliopathy (elevated syndecan-1 and vascular endothelial cadherin levels), sympathoadrenal activation (elevated epinephrine levels), and coagulopathy (both hypo- and hypercoagulable states) on rapid thromboelastography.[41] High epinephrine, syndecan-1, and vascular endothelial cadherin levels, as well as other clinical parameters, were independent predictors of hypocoagulopathy, as measured by rapid thromboelastography. Additionally, other markers indicative of endothelial activation (circulating E-selectin), as well as transfusion of plasma in the prehospital setting, showed an association with parameters of hypercoagulability on rapid thromboelastography. These findings support the close interplay among EGL,

Table 3. Multivariable Logistic Regression Model of Variables Associated with 30-Day In-Hospital Mortality in 407 Trauma Patients Admitted to a Level I Trauma Center

Variable	Adjusted odds ratio (95% CI)*	p Value
Endotheliopathy of trauma-positive	2.23 (1.22−4.04)	0.009
Age, 1 y	1.04 (1.03−1.05)	<0.001
Injury Severity Score, 1 point	1.04 (1.01−1.07)	0.01
Arrival systolic blood pressure, 1 mmHg	1.01 (1.00−1.02)	0.01
Base excess, 1 mEq/L	0.96 (0.91−1.02)	0.27

*Adjusted odds ratios are interpreted as changes in the odds per unit in the predictor, for these continuous variables, after adjusting for the other variables in the model. Age, Injury Severity Score, arrival systolic blood pressure, and base excess were analyzed in the model as continuous variables.

coagulation, and inflammation triggered by trauma reported previously in several studies, but more importantly, establish an association between coagulopathy measured by rapid thromboelastography, sympathoadrenal activity, and endothelial dysfunction in trauma patients.[3,16,19,20,42]

Our study has several limitations. The single-center design and relatively small sample size are most notable. Additionally, because this study was observational, it cannot provide insight into cause and effect mechanisms, but simply points toward associations that require additional study. Because there is no definitive quantitative marker of EoT, and several mechanisms (some yet to be identified) are expected to be involved in this syndrome, using only syndecan-1 and sTM as quantitative markers of EoT represents a limitation in and of itself. However, both are well-described biomarkers of EGL and endothelial cell injury, and several studies have demonstrated the role of syndecan-1 in endothelial damage and disruption, and the succeeding downstream effects.[2,3,10,29,31]

CONCLUSIONS

EoT as defined (syndecan-1 level $\geq$40 ng/mL) is a syndrome associated with an increased requirement for transfusion of blood products, longer hospital stay, and lower survival rate in the absence of clinically significant differences in admission physiology. The prompt identification of this population is important to develop clinical studies that allow early diagnosis and treatment of EoT, that is, patients in need of endothelial repair. Clinically, timely interventions aimed at protecting and repairing the endothelium and its EGL could attenuate or prevent traumatic endotheliopathy and potentially improve outcomes, but this warrants additional research.

Author Contributions

Study conception and design: Gonzalez Rodriguez, Ostrowski, Cardenas, Wade

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Analysis and interpretation of data: Gonzalez Rodriguez, Ostrowski, Henriksen, Stensballe, Cotton, Holcomb, Johansson, Wade

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